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### Data Article

# Data in support of quantification of pyrophosphate as a universal approach to determine polymerase activity and assay polymerase inhibitors



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### ABSTRACT

Characterization of synthetic oligonucleotides and quantification of primer extension mediated by a human translesion synthesis polymerase  $\eta$  (Pol  $\eta$ ) over drug-induced DNA lesions in the presence of modified nucleotide analogs is described. Extent of primer extension for each reaction was monitored by denaturing gel electrophoresis. The data was obtained to assess the performance of the fluorescence-based primer extension (PE-PiPer) assay [1] with respect to the established and conventionally used denaturing gel electrophoresis. The obtained data reflects the specific inhibition of translesion synthesis over cisplatin containing DNA with 5-OH-CTP.

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## Specifications table

Subject area	Chemical Biology
More specific subject area	Toxicology, Drug Development
Type of data	Table, text file, graph, figure
How data was acquired	Oligonucleotide synthesis, fluorescence assay, gel electrophoresis
Data format	Analyzed by Mass Spectrometry, Fluorescence plate-reader, and Molecular Imager Gel Doc XR+ Imaging System from Bio-Rad
Experimental factors	Enzymes were contained in Tris buffer.
Experimental features	Fluorescent measurements were used to evaluate DNA synthesis. The data was correlated to that obtained with 1D gel electrophoresis.
Data source location	Zurich, Switzerland
Data accessibility	Data is provided in <a href="#">Supplementary materials</a> directly with this article.

## Value of the data as follows:

- Quantitative analysis of translesion synthesis over DNA with drug-relevant adducts.
- Quantitative evaluation of DNA synthesis inhibition and identification of lesion-specific translesion synthesis inhibitors.

## 1. Data, experimental design, materials and methods

We have established a fluorescence-based assay for quantitative monitoring of DNA synthesis based on the formation of pyrophosphate [1]. The assay was developed for the use with DNA containing different DNA alkylation products. DNAs were analyzed with Mass Spectrometry and the corresponding spectra are shown in [Supplementary materials](#). Replication of modified and natural DNA templates by Y-family translesion synthesis polymerases was monitored and quantified based on fluorescence emission being proportional to pyrophosphate release. The data were correlated to those produced by monitoring replication of modified and natural DNA templates via gel electrophoresis, and the comparative data are shown in [Supplementary materials](#). The data support the reliability of the assay and its applicability to high throughput screening of polymerase inhibitors. Validation of the assay with unnatural nucleosides resulted in the observation that lesion-specific inhibition of translesion synthesis is possible.

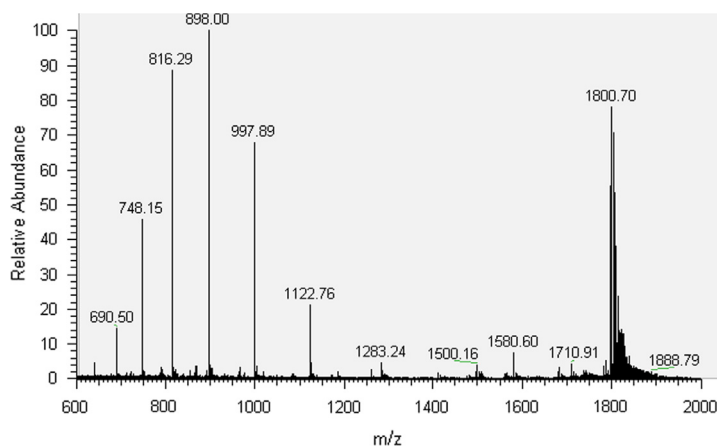


Fig. 1. MS spectrum of 30mer-GG.

1.1. Oligonucleotide characterization

The synthesized oligonucleotides were purified by HPLC and characterized by MS analysis (Supplementary material, Figs. 1–4). 30mer-GG and 30mer-*O*<sup>6</sup>-MeG DNA templates were purified by HPLC (Agilent 1100 Series) with an Agilent Eclipse XDB-C18 5  $\mu$ m 4.6  $\times$  150 mm<sup>2</sup> column. The chromatographic mobile phases were 50 mM triethylammonium acetate and acetonitrile. A gradient of acetonitrile from 8 to 12% over 30 min was used for 30mer-GG and from 9.5 to 12% over 49 min for 30mer-*O*<sup>6</sup>-MeG. For 30mer-Pt, a Phenomenex Luna C18 5  $\mu$ m 250  $\times$  4.6 mm<sup>2</sup> column was used with an acetonitrile gradient from 5 to 12% over 75 min. The eluted fractions containing DNA templates were

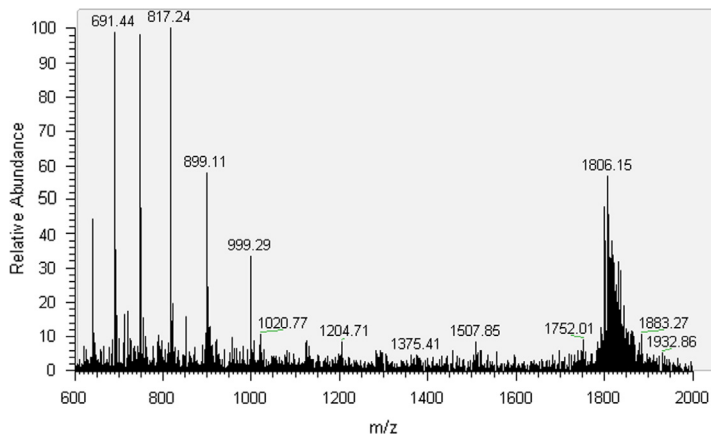


Fig. 2. MS spectrum of 30mer-*O*<sup>6</sup>MeG.

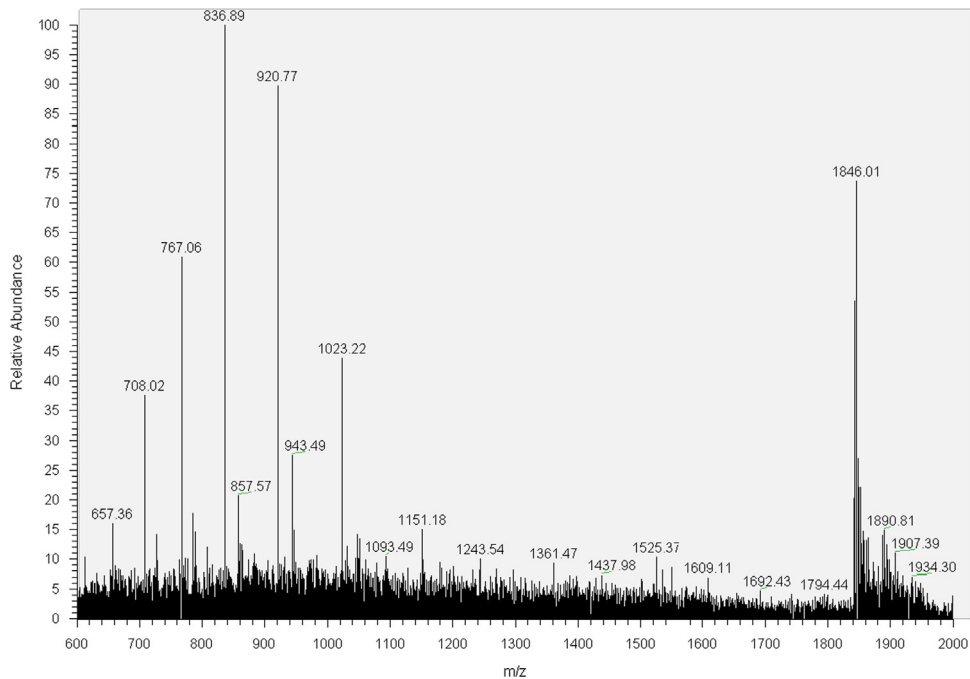


Fig. 3. MS spectrum of 30mer-Pt.

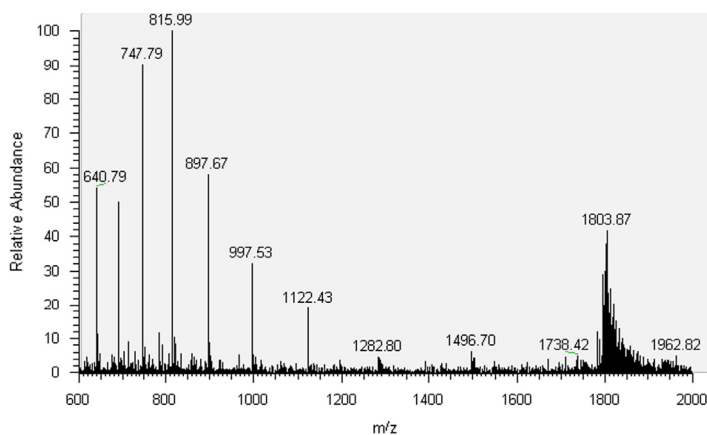


Fig. 4. MS spectrum of 30mer-3d-3MeA.

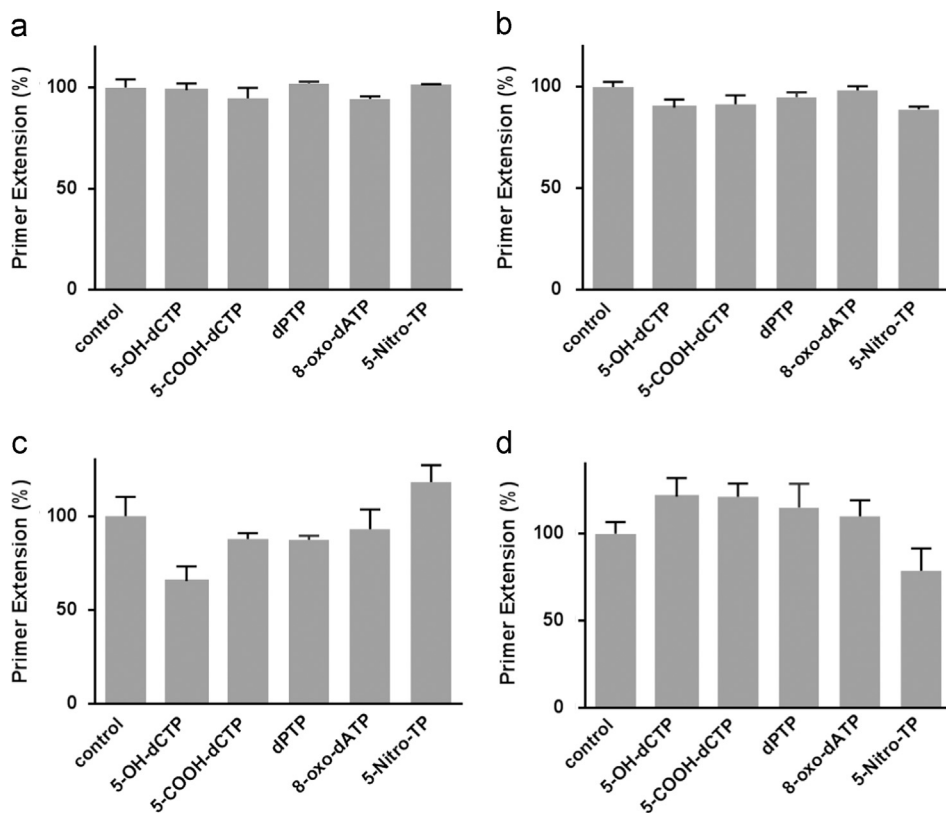


Fig. 5. Modulation of Pol  $\eta$  TLS activity by nucleotide analogs detected by gel electrophoresis. Reaction solutions contained 20 nM Pol  $\eta$ , 600  $\mu$ M dNTPs and 60  $\mu$ M nucleotide analogs. DNA templates (1.6  $\mu$ M) were 30mer-GG (a), 30mer-O6MeG (b), 30mer-Pt (c) and 30mer-3d-3MeA (d). Graph bars represent the average of independent replicates and their standard deviation ( $n=3$ ).

concentrated to dryness in a MiVac centrifugal evaporator (GeneVac), resuspended in deionized water and checked by direct injection into an Agilent MSD SL ion trap mass spectrometer with electrospray ionization. The template 30mer-3d-3MeA was purified by PAGE electrophoresis with a 20% (w/v) acrylamide/urea 7 M gel followed by solid phase extraction with a Sep-Pak C18 Classic cartridge (Waters).

### 1.2. Gel electrophoresis data

Extent of primer extension for each reaction was monitored in parallel by denaturing gel electrophoresis for comparison with PE-PiPer data (Fig. 5). 2 µl of the quenched primer extension reaction were combined with 3 µl loading buffer (95% formamide, 18 mM EDTA, 0.1% Bromophenol Blue) and electrophoresis was performed with a 20% (w/v) acrylamide/urea 7 M gel with an XCell SureLock mini-cell electrophoresis system from Life Technologies at 300 V for 90 min. The gel was then stained in 1X SYBR Gold solution at room temperature for 20 min. Gel bands were quantified with a Molecular Imager Gel Doc XR+ Imaging System from Bio-Rad.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.dib.2015.04.006>.

## References

- [1] S. Malvezzi, S.J. Sturla, M. Tanasova, Quantification of pyrophosphate as a universal approach to determine polymerase activity and assay polymerase inhibitors, *Anal. Biochem.* 478 (2015) 1–7, <http://dx.doi.org/10.1016/j.ab.2015.03.002>.